Wisconsin State Laboratory of Hygiene

UNIVERSITY OF WISCONSIN–MADISON

How to Modify an FDA Approved Test without Really Trying----------and the Consequences

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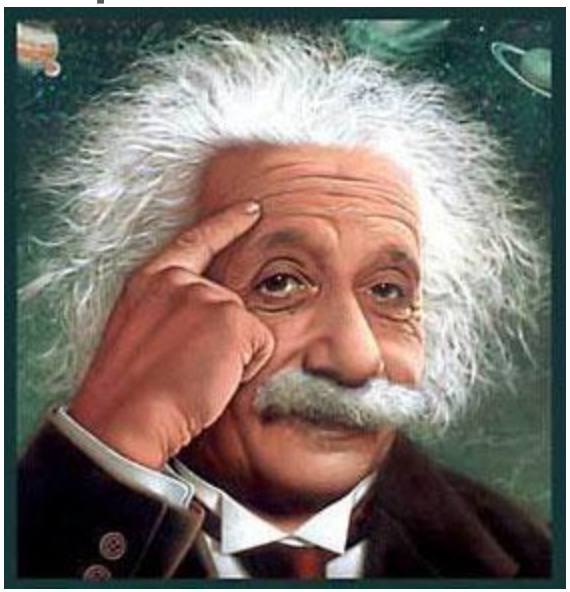
My Laboratory's policy is

A. that we use ONLY FDA approved/cleared molecular tests

B. that we can use laboratory developed molecular tests

Modification of an FDA approved test

"Modified by the laboratory" means any change to an assay that could affect its performance specifications



Modification of an FDA approved test

- Using a specimen type not included in package insert
- Changing the extraction method
- Using a different collection device
- Changing specimen handling instructions
- Changing the cutoff value or method of calculation

CLIA Requirements

After April 24, 2003, any new high complexity test introduced into the laboratory must be verified

- Laboratory developed test
- Modification of the manufacturer's test procedure
- Any non-FDA cleared method

Terminology
or
"What's in a name? That which we call a rose By any other name would smell as sweet."

- **Verification***
  - The documentation of either commercial or laboratory developed tests to determine or confirm test performance characteristics before the test system is used for patient testing
    - A one-time process

*Cumitech 31A
*CLSI MM3-A2

Terminology

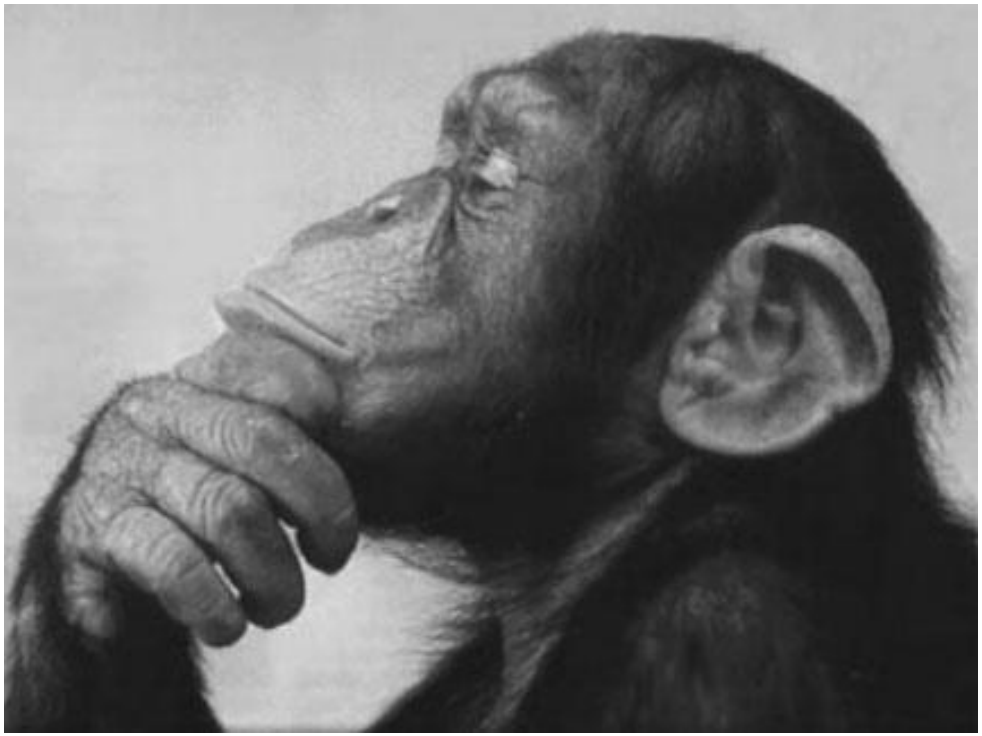
Validation

- The documentation that a test which has already been **verified** is repeatedly giving the expected results as the test is performed over a period of time.
  - **Quality control**
  - **Proficiency testing**
  - **Validation of employee competency**
  - **Instrument calibration**
  - **Correlation with clinical findings**

Purposes of Method Verification

- To quantifiably characterize test performance
- To assess the potential for error
- To identify method-to-method differences
- To meet regulatory guidelines

Verification Study Design for Laboratory Developed Tests (LDTS)



Does your laboratory have a policy and procedure for method verification?

A. Yes

B. No

C. If we do, I've never seen it

CLIA Performance Characteristics Section 493.1253

CLIA specifies performance characteristics----- but not how to do it or criteria to be used

- Accuracy
- Precision
- Analytical sensitivity
- Analytical specificity
- Reportable Range
- Reference range(s)
- Any other characteristics required for test performance and interpretation of results

Verification Study Design

- Establish the type and number of specimens necessary
- Decide on a comparative method or "gold standard"
- Acceptance criteria
- Methods for resolving discrepancies

Discrepant Analysis

Discrepancies

- May arise due to errors in the method being evaluated
- Comparative method is not 100% accurate

Resolving discrepancies

- Use a designated reference standard method
- Send to another laboratory that uses a different method
- Use a test that targets another area of the gene

What Types of Samples can be used?

Should be typical of those that will be routinely tested

- Patient samples with known results
  - Retention specimens
  - Specimens from another laboratory

Other Options

- Quality control material
- Proficiency testing samples
- Calibration material
- Spiked negative patient specimens
- Manufacturer's verification panels???

PERFORMANCE CHARACTERISTICS FOR VERIFICATION

Analytical Accuracy

Closeness of an individual measurement to the "true" value, as determined by a reference method

Numbers of known positive and negative specimens should be balanced or statistically significant to have confidence in the test result

- e.g. ≥50 positive, ≥50 negative
- Confidence interval of 78-97% (CLSI EP12)

$$\frac{\text{Number of correct results}}{\text{Total number of results}} \times 100$$

Side-by-Side Comparison of Real-Time PCR Assay to Gen Probe® Amplified™ MTD Test at WSLH

Number of Specimens	MTD Positive	MTD Negative	MTD Inhibited
Real-Time PCR Positive	49	1	2
Real-Time PCR Inhibited	0	0	0
Real-Time PCR Negative	0	50	3

Accuracy = (99/100) x 100 = 99%

1 "Incorrect" Result

<u>Discrepant Analysis</u>

- A different TB NAAT
- Culture Result

Analytical Sensitivity (Limits of Detection)

LoD---the lowest amount of target that can be detected by a test system with a stated probability

Methods

- Spiking whole organisms into negative specimens
  - Known CFUs/ml, PFUs/ml, $TCID_{50}$/ml
- Spiking with known number of copies of the target

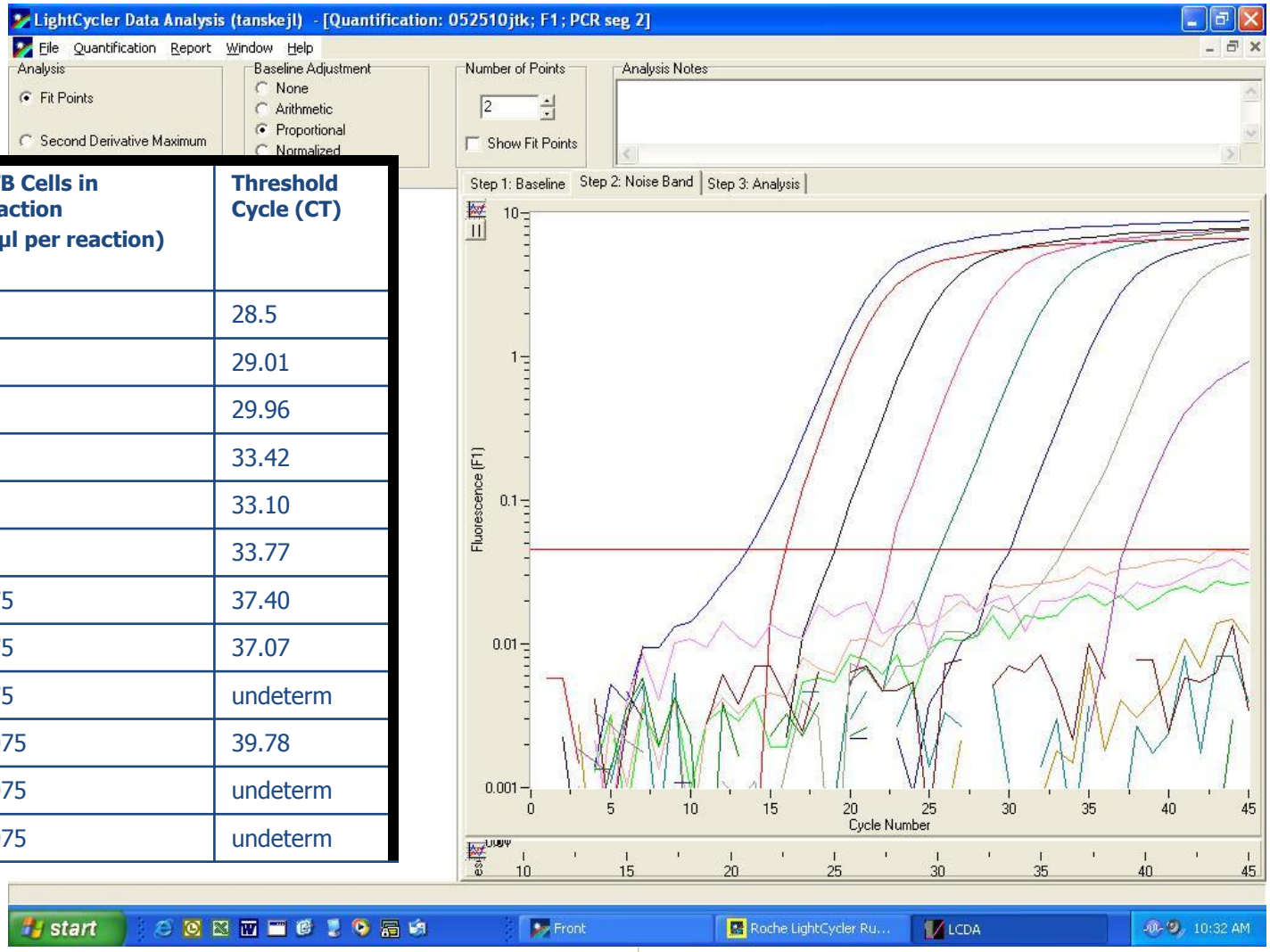
Evaluate for the influence of microbial diversity

- Different patient isolates, serogroups, serotypes, lineages, resistance phenotypes, etc.

Reference---EP17-A: Protocols for Determination of Limits of Detection and Quantitation

Anaytical Sensitivity

MTB Cells in Reaction (5 µl per reaction)	Threshold Cycle (CT)
75	28.5
75	29.01
75	29.96
7.5	33.42
7.5	33.10
7.5	33.77
0.75	37.40
0.75	37.07
0.75	undeterm
0.075	39.78
0.075	undeterm
0.075	undeterm



Cell Suspension	Mtb/Reaction	Threshold Cycle
1.5×10^3	7.5	32.98
	7.5	33.57
	7.5	33.81
	7.5	32.89
	7.5	32.96
	7.5	31.9
	7.5	33.2
	7.5	30.9
	7.5	33.00
	7.5	31.45
	7.5	33.57
	7.5	32.48
	7.5	31.75
	7.5	30.12
	7.5	32.58
	7.5	33.42
	7.5	31.69
	7.5	33.01
	7.5	32.79
	7.5	32.51
	Average	**33.11**

1.5×10^2	0.75	36.47
	0.75	36.12
	0.75	35.70
	0.75	35.70
	0.75	34.38
	0.75	35.77
	0.75	38.38
	0.75	35.88
	0.75	36.86
	0.75	37.45
	0.75	35.22
	0.75	36.13
	0.75	37.13
	0.75	35.56
	0.75	37.62
	0.75	38.10
	0.75	37.00
	0.75	36.51
	0.75	Undeterm
	0.75	36.08
	Average	36.42

LoD

Analytical Specificity

Ability of a method to detect only the intended target

Verify for cross-reactivity

- Organisms closely related to the target organism
- Organisms that represent normal flora of the specimen being tested
- Organisms that cause similar disease syndromes

Can use whole organisms that go thru the complete method and/or extracted nucleic acids

Specificity and Breadth of Detection

Negative PCR Result		Positive PCR Result
M. avium complex ATCC700898		
M. gordonae ATCC 1218	*Tsukamurella* sp.	MTBC ATCC 27294
M. xenopi		
M. scrofulaceum	*L. pneumophila*	MTBC ATCC 35828
M. peregrinum ATCC 700686	*N. meningitidis*	
M. smegmatis ATCC 1546	*S. pneumoniae*	32 TB patient isolates and 3 *M. bovis* BCG isolates
M. marinum	Group A *Streptococcus*	
M. mucogenicum	*H. influenzae*	
M. chelonae	*P. aeruginosa*	
M. abscessus	*K. pneumoniae*	
M. fortuitum ATCC 1447	*B. Pertussis*	
M. kansasii ATCC 12478	*B. parapertussis*	

Interfering Substances

- Blood
  - Lipemic
  - Icteric
  - Hemolyzed
  - Anticoagulants (EDTA, heparin, ACD)
- Sputum
  - Bloody
  - Decontamination process

Precision (Reproducibility)

Within run

Run-to-run, same day

Day-to-day, different analysts

Inter-instrument

Protocol described in CLS EP12

- e.g. For a qualitative assay interpreted from a quantitatively measured signal-----Calculate SD and CV from 10-20 day-to-day quality control results
- Test 2 concentrations in duplicate, 2x/day for 20 days
- Run aliquots of a single specimen 30 times in a single run
- Panel of samples run by different analysts

Goal of 95% typical for PCR assays

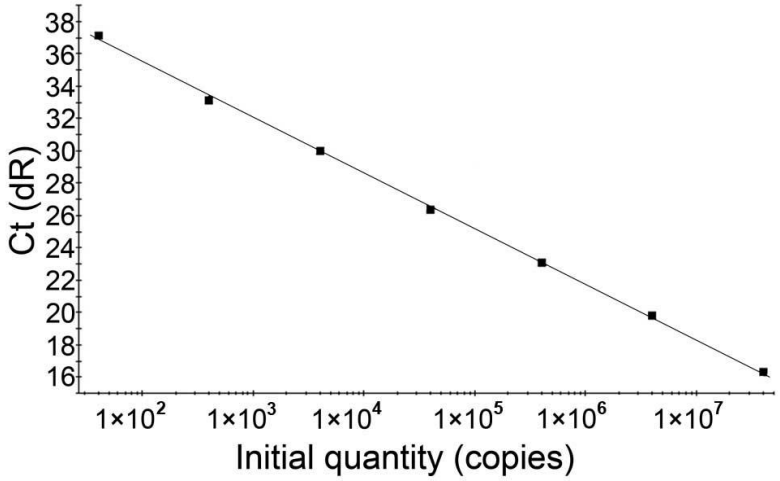
Reportable Range

For qualitative assay---Not Applicable

- Detected or not detected

For quantitative assay

- The range of results for which a test has been proven to yield numerically accurate results. (CLSI Document EP17-A)



Verification Documentation

Write up

- Purpose and Background
  - References for clinical utility
  - Intended use of the assay
  - Specimen types and matrixes
- Methods
- Results
- Conclusions

Review, approval, sign off by Laboratory Director or designee who qualifies as a Director

Save for $\geq$ 2 years after test is discontinued

FDA-Cleared Test Verification

If unmodified

- Accuracy
- Precision
- Reportable range
- Reference range

Don’t have to assess sensitivity and specificity

Accuracy and precision should fall within manufacturer’s specifications

- Can use fewer specimens

Public Health Laboratory Exceptions

Exceptions for PHLs when calibration or control materials are not available

- e.g. During public health emergencies, tests for emergent diseases, or public health threats
- Emergency Use Authorization (EUA)

Public Health Laboratory Exceptions con't

Given temporary CLIA exemption

- Must follow CDC protocols w/o modification
- Personnel must show proficiency in the test method
- Must document alternative methods to show accuracy
  - Send a number of samples to CDC for verification
  - Test with another method

Must do complete validation when calibration material is available or EUA expires

Summary

Verification studies are a "Necessity of Laboratory Life"

Verification studies require a major time commitment

Verification studies can be a major expense

Verification studies must be carefully designed

- Important to utilize the guidelines that are available

References

CLSI document MM3-A2. Molecular Diagnostic Methods for Infectious Diseases.

CLSI document MM6-A. Quantitative Molecular Methods for Infectious Diseases.

CLSI document MM13-A. Collection, Transport, Preparation, and Storage of Specimens for Molecular Methods.

CLSI document MM17-A. Verification and Validation of Multiplex Nucleic Acid Assays.

CLSI document EP12-A2. User Protocol for Evaluation of Qualitative Test Performance.

References

CLSI document EP17-A. Protocols for Determination of Limits of Detection.

CLSI EP05-A2 document. Evaluation of Precision Performance of Quantitative Measurement Methods.

CUMITECH 31. Verification and Validation of Procedures in the Clinical Microbiology Laboratory. ASM Press

National Laboratory Training Network Verification of Infectious Disease Molecular Assays. August 2005. Copies available www.nltn.org

References

CLSI document GP27-A2. Using Proficiency Testing to Improve the Clinical Laboratory.

CLSI document GP29-A. Assessment of Laboratory Tests When Proficiency Testing is Not Available.

Thank you!